10/001688?

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=> s (urea or acetamide or acrylamide or arylamide or
aralkylamide)(10a)(hybridiz##### or PCR)
L1 650 (UREA OR ACETAMIDE OR ACRYLAMIDE OR ARYLAMIDE OR ARALKYLAMIDE)(1
0A)(HYBRIDIZ##### OR PCR)

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L2 0 L1 AND TEMPERTURE#

=> s l1 and temperature#
L3 14 L1 AND TEMPERATURE#

=> s l3 and heat###
L4 0 L3 AND HEAT###

=> dup rem l3
PROCESSING COMPLETED FOR L3
L5 7 DUP REM L3 (7 DUPLICATES REMOVED)

=> d l5 1-7 bib ab kwic

L5 ANSWER 1 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2002:356362 BIOSIS
DN PREV200200356362
TI Stabilized viral nucleic acids in plasma as an alternative shipping method for NAT.
AU Lee, Dong-Hun; Li, Liping; Andrus, Linda; Prince, Alfred M. (1)
CS (1) Laboratory of Virology, New York Blood Center, 310 East 67th Street, New York, NY, 10021: aprince@nybc.org USA
SO Transfusion (Bethesda), (April, 2002) Vol. 42, No. 4, pp. 409-413. print. ISSN: 0041-1132.
DT Article
LA English
AB BACKGROUND: Preservation of the integrity of viral nucleic acids in blood specimens during shipping and handling is crucial for NAT and viral load monitoring. An economical and convenient method is described for nucleic acid stabilization by using an RNA stabilizing solution (RNAlater, Ambion) in plasma that is designed for the shipment of samples to tropical countries. STUDY DESIGN AND METHODS: HCV, HIV, and HBV FFP were compared with RNAlater-treated plasma and dried plasma spots (DPSs) after incubation at 37degreeC, which was chosen as an upper limit of ambient shipping **temperature**, for up to 28 days. HCV-infected chimpanzee plasma was shipped at either room **temperature** after RNAlater treatment or as frozen plasma in liquid nitrogen from Liberia to New York City. They were then compared for HCV RNA levels. The nucleic acid stabilities were determined by quantitative PCR by using a molecular beacon assay on a sequence detection system (ABI 7700, PE-Biosystems) and by visualizing the **PCR** components on an **acrylamide**

gel. RESULTS: Quantitative **PCR** data showed that a 60:40 or greater ratio of RNAlater:plasma volume successfully stabilized HCV RNA and HIV RNA in plasma for up to 28 days at 37degreeC. HBV DNA in plasma was stable for up to 14 days at 37degreeC without any stabilizing solution. DPSs on filter paper stabilized viral nucleic acids, but the recoveries were 3 to 10 times less than those with frozen plasma. The integrity of the 5' UTR region of HCV RNA in RNA later-treated chimpanzee plasma was intact when its **PCR** component was viewed on an **acrylamide** gel. CONCLUSION: The DPS method stabilized nucleic acids, at least with the extraction method used, was less sensitive than use of RNAlater, and required tedious manual handling. RNAlater provides a convenient way of stabilizing viral nucleic acid in plasma at ambient **temperature** during sample transportation.

AB. . . plasma and dried plasma spots (DPSs) after incubation at 37degreeC, which was chosen as an upper limit of ambient shipping **temperature** , for up to 28 days. HCV-infected chimpanzee plasma was shipped at either room **temperature** after RNAlater treatment or as frozen plasma in liquid nitrogen from Liberia to New York City. They were then compared. . . quantitative PCR by using a molecular beacon assay on a sequence detection system (ABI 7700, PE-Biosystems) and by visualizing the **PCR** components on an **acrylamide** gel. RESULTS: Quantitative **PCR** data showed that a 60:40 or greater ratio of RNAlater:plasma volume successfully stabilized HCV RNA and HIV RNA in plasma. . . plasma. The integrity of the 5' UTR region of HCV RNA in RNA later-treated chimpanzee plasma was intact when its **PCR** component was viewed on an **acrylamide** gel. CONCLUSION: The DPS method stabilized nucleic acids, at least with the extraction method used, was less sensitive than use. . . RNAlater, and required tedious manual handling. RNAlater provides a convenient way of stabilizing viral nucleic acid in plasma at ambient **temperature** during sample transportation.

IT . . .
preservation method; dried plasma spot method: preservation method; quantitative PCR [quantitative polymerase chain reaction]: evaluation method

IT Miscellaneous Descriptors
ambient **temperature**; tropical countries

L5 ANSWER 2 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

AN 2001:373923 BIOSIS

DN PREV200100373923

TI Multiple use of slab gels in sequencing apparatus for separation of polymerase chain reaction products.

AU Man, Yan-Gao (1); Kuhls, Elizabeth A.; Bratthauer, Gary L.; Moinfar, Farid; Tavassoli, Fatteneh A.

CS (1) Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology and American Registry of Pathology, 6825 16th Street, N. W., Washington, DC, 20306-6000: man@afip.osd.mil USA

SO Electrophoresis, (June, 2001) Vol. 22, No. 10, pp. 1915-1919. print. ISSN: 0173-0835.

DT Article

LA English

SL English

AB Attempting to assess whether a decrease of the electrophoresis **temperature** could prevent or reduce the extent of gel well deformations, and whether the utilization of native polyacrylamide gels (without **urea**) could speed up the separation of polymerase chain reaction (**PCR**)-amplified products with an automated 377 DNA sequencer, denatured PCR products were subjected to electrophoresis in 6% native gels under 45degreeC. Results show that a decrease of the electrophoresis **temperature** from 51degreeC (recommended by the User's Manual) to 45degreeC substantially facilitates the preservation of gel wells, and that all PCR products tested migrate significantly faster in native than in denatured (with urea) gels of the same concentration.

The combination of a 6% native gel and a lower (45degreeC) electrophoresis **temperature** permits multiple uses of a given gel with consistent results, consequently reducing the electrophoresis time and reagent costs.

AB Attempting to assess whether a decrease of the electrophoresis **temperature** could prevent or reduce the extent of gel well deformations, and whether the utilization of native polyacrylamide gels (without **urea**) could speed up the separation of polymerase chain reaction (**PCR**)-amplified products with an automated 377 DNA sequencer, denatured PCR products were subjected to electrophoresis in 6% native gels under 45degreeC. Results show that a decrease of the electrophoresis **temperature** from 51degreeC (recommended by the User's Manual) to 45degreeC substantially facilitates the preservation of gel wells, and that all PCR. . . denatured (with urea) gels of the same concentration. The combination of a 6% native gel and a lower (45degreeC) electrophoresis **temperature** permits multiple uses of a given gel with consistent results, consequently reducing the electrophoresis time and reagent costs.

IT . . .
Molecular Biology Techniques and Chemical Characterization, separation method; sequencing apparatus: laboratory equipment; slab gels: laboratory equipment

IT Miscellaneous Descriptors
electrophoresis **temperature**; gel well deformations; reagent costs

L5 ANSWER 3 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:431866 BIOSIS
DN PREV199900431866
TI Human mitochondrial DNA analysis by fluorescence based-polymerase chain reaction-single strand conformation polymorphism (F-PCR-SSCP) at hypervariable portion I (HV1) in control region.
AU Kim, Jong-Jin; Choi, Sang-Kyu; Lee, Hyeong-Hwan
SO Korean Journal of Genetics, (Sept., 1999) Vol. 20, No. 3, pp. 191-202. ISSN: 0254-5934.
DT Article
LA Korean
SL English; Korean

AB Human mitochondrial DNA (mtDNA) was analyzed by fluorescence based-polymerase chain reaction-single strand conformation (F-PCR-SSCP) at nucleotide positions between 16261 and 16380 of hypervariable portion I (HV1) in the control region. The best condition of F-PCR-SSCP analysis for mutation separation was identified when the **PCR** product of mtDNA was electrophoresed on 6% polyacrylamide (49:1 **acrylamide**:bis) gel containing 8% glycerin at a constant gel **temperature** of 20degreeC for 8 hours. mtDNA SSCP patterns were divided into 7 groups according to mobility shift differentiation of PCR products from 100 Koreans by F-PCR-SSCP analysis, and the groups could be again classified into forty-seven mtDNA SSCP patterns when the mtDNA SSCP patterns were compared with the data of DNA sequencing. When 6 unidentified human remains from a mass disaster were examined family relationships by F-PCR-SSCP analysis, mtDNA SSCP pattern of every human remanis was identical with the pattern of mother. So, it wasdemonstrated F-PCR-SSCP of mtDNA to be very useful technique for individual identification of forensic samples.

AB. . . portion I (HV1) in the control region. The best condition of F-PCR-SSCP analysis for mutation separation was identified when the **PCR** product of mtDNA was electrophoresed on 6% polyacrylamide (49:1 **acrylamide**:bis) gel containing 8% glycerin at a constant gel **temperature** of 20degreeC for 8 hours. mtDNA SSCP patterns were divided into 7 groups according to mobility shift differentiation of PCR. . .

L5 ANSWER 4 OF 7 MEDLINE
AN 96401744 MEDLINE

DN 96401744 PubMed ID: 8964639
TI Predilution hemofiltration. Clinical experience and removal of small molecular weight solutes.
AU David S; Bostrom M; Cambi V
CS Department of Nephrology, University of Parma, Italy.
SO INTERNATIONAL JOURNAL OF ARTIFICIAL ORGANS, (1995 Nov) 18 (11) 743-50. Journal code: 7802649. ISSN: 0391-3988.
CY Italy
DT (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
ED Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961205
AB Over 1500 treatments of hemofiltration with on-line preparation of substitution fluid were performed in 16 patients. Two patients were treated for over 40 months. On-line preparation of the solution allowed use of bicarbonate as a buffer. 73-74 L/session were infused in pre-dilution modality, at a rate of about 370 ml/min, and the treatment length was above 4 hrs. The good quality of on-line prepared solution was confirmed by the negativity of microbiological tests and by the absence of clinical or sub-clinical reactions in patients. Urea clearance was calculated by equations considering either plasma flow or whole blood flow. Results were 196-197 ml/min and 186-183 ml/min, respectively. The latter was nearer to the value of directly measured clearance (182-173 ml/min). Kt/V **urea** was about 1 per session and **PCR** ranged between 1.3 and 1.4 g/kg/day. A high vascular stability was also observed. Since sodium balance may, at least in part, account for better vascular stability, sodium sieving coefficient was measured during the treatment. The sodium-retaining effect of the increase of protein concentration within the filter, due to the ultrafiltration, was less relevant in pre-dilution hemofiltration if compared to post-dilution hemofiltration. It has been calculated that to obtain a sodium balance similar to that of the hemodialysis (HD), the sodium concentration of infusion solution should be about 2 mEq/L higher than HD dialysis solution. However, difficulty in performing accurate balance studies prevents a general agreement on these conclusions.
AB . . . 196-197 ml/min and 186-183 ml/min, respectively. The latter was nearer to the value of directly measured clearance (182-173 ml/min). Kt/V **urea** was about 1 per session and **PCR** ranged between 1.3 and 1.4 g/kg/day. A high vascular stability was also observed. Since sodium balance may, at least in. . .
CT Check Tags: Comparative Study; Female; Human; Male
Acetates: BL, blood
Bicarbonates: BL, blood
Blood Pressure: PH, physiology
Body Temperature: PH, physiology
Body Weight: PH, physiology
Chlorides: BL, blood
*Electrolytes: BL, blood
Glucose: ME, metabolism
Heart Rate: PH,. . .

L5 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
AN 1995:528038 BIOSIS
DN PREV199598542338
TI Downward blotting of proteins in a model based on apolipoprotein(a) phenotyping.
AU Naby, Balint; Costello, Rene; Csako, Gyorgy (1)
CS (1) Clinical Pathol. Dep., Warren G. Magnuson Clinical Center, National Inst. Health, Building 10, Room 2C-407, Bethesda, MD 20892 USA
SO Analytical Biochemistry, (1995) Vol. 231, No. 1, pp. 40-45.

ISSN: 0003-2697.
DT Article
LA English
AB Standard immunoblotting ("Western blot") involves electrotransfer of proteins from a separation gel (usually **acrylamide**) onto a membrane. Recently, a downward capillary method with increased **hybridization** efficiency was developed for DNA and RNA. The present work assessed the applicability of this method to proteins in a model based on human apolipoprotein(a) (apo(a)) isoforms which consist of a single, gt 200-kDa polypeptide chain varying in size with a repeat sequence. After reduction treatment and sodium dodecyl sulfate-agarose gel electrophoresis, serum proteins were transferred from the gel by upward or downward (Turboblotter) capillary action onto nitrocellulose membranes in Tris-buffered saline, pH 7.5, at room **temperature**. Increased detectability of apo(a) isoforms was achieved by substituting comparatively high molar concentrations of protein A for true second antibody. With downward capillary transfer and short 37 degree C incubations, the apo(a) phenotyping could be completed in about 26 h and required less than 8 h effective processing time. The downward transfer was about twice as fast (complete within 1 h) as the upward version and with this speed it offers a good alternative to electroblotting as well.
AB Standard immunoblotting ("Western blot") involves electrotransfer of proteins from a separation gel (usually **acrylamide**) onto a membrane. Recently, a downward capillary method with increased **hybridization** efficiency was developed for DNA and RNA. The present work assessed the applicability of this method to proteins in a. . . from the gel by upward or downward (Turboblotter) capillary action onto nitrocellulose membranes in Tris-buffered saline, pH 7.5, at room **temperature**. Increased detectability of apo(a) isoforms was achieved by substituting comparatively high molar concentrations of protein A for true second antibody.. . .

L5 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2002 ACS
AN 1972:431241 CAPLUS
DN 77:31241
TI DNA-DNA hybridization on filters at low **temperature** in the presence of formamide or urea
AU Kourilsky, Ph.; Leidner, J.; Tremblay, G. Y.
CS Cent. Hosp. Univ., Sherbrooke, Que., Can.
SO Biochimie (1971), 53(10), 1111-14
CODEN: BICMBE
DT Journal
LA English
AB Lambda-phage DNA-DNA **hybridization** is carried out on filters in 8M **urea**-2 .times. (0.15M NaCl-0.015M Na citrate, pH 7) or 50% formamide-2 .times. (0.15M NaCl-0.015M Na citrate, pH 7.0) at 40.degree. with results comparable to 2 .times. (0.15M NaCl-0.015M Na citrate, pH 7.0) alone at 65.degree..
TI DNA-DNA hybridization on filters at low **temperature** in the presence of formamide or urea
AB Lambda-phage DNA-DNA **hybridization** is carried out on filters in 8M **urea**-2 .times. (0.15M NaCl-0.015M Na citrate, pH 7) or 50% formamide-2 .times. (0.15M NaCl-0.015M Na citrate, pH 7.0) at 40.degree. with results comparable to 2 .times. (0.15M NaCl-0.015M Na citrate, pH 7.0) alone at 65.degree..
IT Deoxyribonucleic acids
RL: ANST (Analytical study)
(**hybridization** of, with DNA on filters in formamide or **urea** presence at low temp.)

L5 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 1971:70772 BIOSIS
DN BR07:70772
TI DNA RNA **HYBRIDIZATION** AT LOW **TEMPERATURE** IN THE

PRESENCE OF **UREA**.
AU KOURILSKY P; MANTEUIL S; ZAMANSKY M H; GROS F
SO Biochem. Biophys. Res. Commun., (1970) 41 (4), 1080-1087.
CODEN: BBRCA9. ISSN: 0006-291X.
FS BR; OLD
LA Unavailable
TI DNA RNA **HYBRIDIZATION** AT LOW **TEMPERATURE** IN THE
PRESENCE OF **UREA**.

=>

10/001,652

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L14	L13 and temperature and heat$3	1	L14
L13	L12 and (urea or acetamide or polyacrylamide or acrylamide or arylamide or arakylamide)	1	L13
L12	6300070.pn.	2	L12
L11	L10 and low$2	5	L11
L10	L9 and hybridiz$5 temperature	5	L10
L9	L8 and (buffer$1 or solution or medium)	153	L9
L8	(urea or acetamide or acrylamide or polyacrylamide or arylamide or arakylamide) near5 denatur$3 near5 (hybridiz$5 or PCR)	157	L8
L7	L6 and (buffer or solution or medium)	143	L7
L6	L5 and hybridization temperature$1	143	L6
L5	L4 and (temperature near5 low$2)	1005	L5
L4	(urea or acetamide or acrylamide or polyacrylamide or arylamide or arakylamide) same (hybridiz$5 or PCR)	4742	L4
L3	(urea or acetamide or acrylamide or arylamide or aralkylamide) near 10 (hybridiz$5 or polymerase chain reaction$1)	0	L3
L2	L1 and (urea or acetamide or acrylamide or arylamide or aralkylamide)	0	L2
L1	6465183.pn.	1	L1

END OF SEARCH HISTORY

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1. 6361940 . 01 Apr 98; 26 Mar 02. Compositions and methods for enhancing hybridization and priming specificity. Van Ness; Jeffrey, et al. 435/6; 435/91.1 435/91.2 536/22.1 536/23.1 536/24.3 536/25.3. C12Q001/68 C07H019/00 C07H021/00 C07H021/02 C07H021/04.

2. 6287854 . 05 Aug 97; 11 Sep 01. Diagnosis of susceptibility to cancer and treatment thereof. Spurr; Nigel K, et al. 435/320.1; 424/280.1 435/252.33 435/325 435/456 435/69.1 530/300 530/350 536/23.5 536/24.31 536/24.33. C12N015/85 C12Q001/68 C07H021/04 C07K005/00.

3. 5773213 . 06 Jun 94; 30 Jun 98. Method for conducting sequential nucleic acid hybridization steps. Gullans; Steven R., et al. 435/6; 435/91.1 435/91.2 536/24.32 536/24.33. C07H021/02 C07H021/04 C12P019/34 C12Q001/68.

4. 5652096 . 20 Apr 94; 29 Jul 97. Identification of allele specific nucleic acid sequences by hybridization with crosslinkable oligonucleotide probes. Cimino; George D.. 435/6; 435/803 435/820 435/91.1 435/91.2 436/501 536/22.1 536/25.3 549/200 549/218 549/275. C12Q001/68 C07H021/00.

5. 5525714 . 08 Oct 93; 11 Jun 96. Mutated form of the .beta.-amyloid precursor protein gene. Van Broeckhoven; Christine, et al. 536/23.5; 536/24.31. C07H021/02 C07H021/04.

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Term	Documents
LOW$2	0
LOW.DWPI,EPAB,JPAB,USPT.	2915267
LOWA.DWPI,EPAB,JPAB,USPT.	45
LOWAC.DWPI,EPAB,JPAB,USPT.	5
LOWAD.DWPI,EPAB,JPAB,USPT.	1
LOWAG.DWPI,EPAB,JPAB,USPT.	18
LOWAI.DWPI,EPAB,JPAB,USPT.	10
LOWAK.DWPI,EPAB,JPAB,USPT.	5
LOWAL.DWPI,EPAB,JPAB,USPT.	1
LOWAN.DWPI,EPAB,JPAB,USPT.	66
LOWAR.DWPI,EPAB,JPAB,USPT.	13
(L10 AND LOW$2).USPT,JPAB,EPAB,DWPI.	5

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L14: Entry 1 of 1 File: USPT Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6300070 B1
TITLE: Solid phase methods for amplifying multiple nucleic acids

US PATENT NO. (1):
6300070

Brief Summary Text (12):
The solid support can be beads, particles, sheets, dipsticks, rods, membranes, filters, fibers (e.g., optical and glass), and the like. Preferably, the solid support is a bead. The material composition of the solid support includes, but is not limited to, plastic, nylon, glass, silica, metal, metal alloy, polyacrylamide, polyacrylate, crosslinked-dextran and combinations thereof. Preferably, the solid support is capable of being modified by the attachment of oligonucleotide primers.

Detailed Description Text (9):
Preferably, the oligonucleotide primers are synthesized such that a modified 5'-acrylamide moiety (Acrydite.TM. phosphoramidite, Mosaic Technologies, Boston, Mass.) is incorporated which will allow the primers to be immobilized within a solid support, for example, a solid support comprising acrylamide. Chemical or photochemical groups subject to cleavage are incorporated into the structure of the linker moieties on the support, or incorporated into one or both primers before immobilization. Additionally, it is possible to introduce cleavable groups during oligonucleotide synthesis in the form of modified phosphoramidites. (Olejnik et al., Nucleic Acid Res., 26:3572-3576 (1998), the teachings of which are herein incorporated by reference in its entirety). The primers are preferably attached to the solid support using covalent interactions. (See Rehman et al., Nucleic Acid Res., 27:649-655 (1999)). However, noncovalent attachment methods can also be practiced with this invention and are well known to those of ordinary skill in the art. (See Cass, T., and Ligler, F. S. (eds), "Immobilized Biomolecules in Analysis: A Practical Approach." 1998. Oxford University Press, Oxford, UK, the entire teachings of which are incorporated herein by reference).

Detailed Description Text (10):
The solid support can be beads, particles, sheets, dipsticks, membranes, filters, fibers (e.g., glass and optical), and the like. Preferably, the solid support is a bead. Suitable material compositions of the solid support includes, but not limited to, plastic, nylon, glass, silica, metal, metal alloy, polyacrylamide, polyacrylates, crosslinked-dextran and combinations thereof. Preferably, the solid support is capable of being modified by the attachment of oligonucleotide primers. The solid support can have any geometric shape. For example, the solid support can approximate a sphere (e.g., a bead). Alternatively, the solid support is planar as a sheet or membrane. The solid support can be magnetic. Preferably, the solid support is thermally stable (e.g., able to withstand temperatures of up to 100.degree. C.) to withstand thermocycling conditions typically used in PCR.

Detailed Description Text (16):
The second double-stranded amplification molecule is subjected to denaturation. Denaturation is effectuated, for example, by placing the second double-stranded amplification molecule in an alkali environment (e.g., 15 mM NaOH). Alternatively, the double-stranded amplification molecule is subjected to melting temperatures which depend upon many factors such as the nucleotide base constituents. Suitable denaturing conditions are well known to those skilled in the art. Following denaturation, the hydrogen bonds between the first and second single-stranded amplification molecules are broken resulting in first and second single-stranded amplification molecules.

These single-stranded molecules still remain attached to the solid support via the oligonucleotide primers. The first single-stranded amplification molecule remains attached via the immobilized first primer; whereas, the second single-stranded amplification molecule remains attached via the immobilized second primer. (See FIG. 3f).

Detailed Description Text (18):
Third and fourth double-stranded amplification nucleic acid molecules (also referred to herein as "third and fourth double-stranded amplification molecules") are formed by contacting the second bridge hybridization complexes with appropriate amplification reagents. (See FIG. 3h). The first and second immobilized primers are extended by the addition of deoxynucleotides. The extension of the second primer uses the first single-standed amplification molecule as a template forming a third single-stranded nucleic acid amplification molecule (also referred to herein as "third single-stranded amplification molecule"). The hybridized first and third single-stranded amplification molecules form a third double-stranded amplification molecule. In a similar manner, the extension of the first primer uses the second single-stranded amplification nucleic acid molecule as a template forming a fourth single-stranded amplification nucleic acid molecule (also referred to herein as "fourth single-stranded amplification molecule"). The hybridized second and fourth single-stranded amplification molecules form a fourth double-stranded amplification molecule. This amplification thermocycle is typically repeated from about five to about fifty cycles generating multiple third and fourth double-stranded amplification molecules. More typically, amplification comprises about thirty-five thermocycles. Each cycle can consist of 95.degree., 60.degree. and 72.degree. C. for about one minute duration for each temperature point. Such thermocycling conditions are well known to those skilled in the art. Additional rounds of thermocycling give rise to a multitude of additional amplification double-stranded molecules.

Detailed Description Text (20):
Methods for cleaving the double-stranded amplification molecule from the solid support other than restriction are also well known to those of skill in the art. For example, chemical cleavage is used if one or both of the two primers are attached to the solid support by a chemical linker that contains a chemically labile group. Dithiol linkages are one example of a linking chemistry that is heat stable but easily cleaved by chemical agents such as dithiothreitol (DTT), .beta.-mercaptoethanol, Tris(2-carboxyethyl) phosphine HCl (TCEP) and other disulfide reducing agents. (Day et al., Biochem. J., 278:735-740 (1991); Singh et al., Methods in Enzymology, 251:167-173 (1995), the teachings of which are herein incorporated by reference in its entirety). Alternatively, photochemical cleavage is employed if one or both of the two primers are attached to the solid support by a linkage moiety that is photochemically labile. Photochemical cleavable attachment chemistries for DNA oligonucleotides have been previously described. (Olejnik et al., Nucleic Acid Res., 26:3572-3576 (1998) and U.S. Pat. No. 5,679,773, the teachings of which are incorporated by reference herein in their entirety). For example, the photochemically cleavable linker can comprise a substituted nitrophenol group.

Detailed Description Text (24):
Denaturation of the cleaved double-stranded amplification molecule is accomplished by exposing the solid support apparatus (i.e., the amplification solid support with attached products) to denaturing conditions, such as high temperatures from about 90.degree. to about 100.degree. C., high pH around 12, or denaturing chemical treatments using organic solvents or chaotropic agents. Alternatively, strand separation is achieved by enzymatic strand-separating methods, for example, treatment of the solid support apparatus with DNA helicases in the presence of ATP. (Lohman, T. M., and Bjornson, K. P., Annu. Rev. Biochem., 65:169-214 (1996), the entire teachings of which are herein incorporated by reference).

Detailed Description Text (32):
This example illustrates a two stage bridge amplification method using a yeast gene fragment (LEU2). The nucleotide sequence of yeast LEU2 gene, bases 7685 to 7943 (Genbank Accession No. AF049063) is shown in FIG. 4. The oligonucleotide primers were synthesized with a 5'-acrylamide modification (Acrydite.TM. phosphoramidite, Mosaic Technologies, Boston, Mass.) which allows the primers to be immobilized to a solid support. In this case, the solid support is a polyacrylamide bead. Copolymerization of

the modified primers with the acrylamide gel mix during bead fabrication produced a solid support with immobilized primers. The primers remained attached via the 5'-acrylamide groups during thermocycling. (Rehman et al., Nucleic Acid Res., 27:649-655 (1999)).

Detailed Description Text (33):
Acrylamide beads with immobilized primers were prepared by pipetting 1 .mu.L drops of a solution containing 10% polyacrylamide (acrylamide/bis, 29:1), 10 mM sodium borate buffer (pH 8.0), 100 .mu.M of each 5'-acrylamide primer (Leu2F2.Pst: 5'-QTT TTT TTT TCT GCA GAA CCG TGG CAT GGT TC-3' [SEQ ID No. 1], and Leu2R3.Xho: 5'QTT TTT TTT TCT CGA GCT GTG GAG GAA ACC ATC AAG-3' [SEQ ID No. 2], restriction sites are italicized, "Q" represents a 5'-acrylamide group, and 0.2% ammonium persulfate (wt/vol) into degassed mineral oil containing 0.4% N,N,N',N'-tetramethylethylenediamine (TEMED). Amplification was allowed to proceed for thirty minutes at room temperature. Excess mineral oil was decanted and the beads were transferred to a 50 mL disposable tube containing 30 mL of TE buffer (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA). The remaining mineral oil was extracted 2 to 3 times using chloroform (15 mL per extraction). The beads were then washed with several rounds of TE buffer (15 mL per round). To remove non-immobilized primers from the beads, the preparation was equilibrated with 0.5.times.TBE (89 mM Tris-borate (pH 8.3) and 2 mM EDTA) and placed into the wells of a vertical polyacrylamide gel, subsequently the preparation was subjected to electrophoresis for sixty minutes at 20 V/cm.

Detailed Description Text (38):
Following restriction digestion of the first stage, 10 .mu.L of the doubly-restricted product were used as the input target nucleic acid molecule for the next stage of bridge amplification. A singly-restricted product was eluted from the PstI treated beads by heating at 94.degree. C. for two minutes. Ten .mu.L of the eluted product were used as the input target nucleic acid molecule for the second stage bridge amplifications. The next stage (in this case, the second stage) hybridization and amplification were performed as that previously described above for the first stage. Products were restricted from the second stage solid supports with RsaI and EcoRI using the same buffer and method described for cleavage of the first stage amplification double-stranded nucleic acid molecules. RsaI and EcoRI cleaved within the double-stranded amplification products. Ten .mu.L aliquots of each reaction were subjected to electrophoresis in a non-denaturing 1.times.TBE, 10% polyacrylamide gel (Novex, San Diego, Calif.). The gel was stained with SYBR green I (Molecular Probes, Eugene, Oreg.) and imaged using a Molecular Dynamics Fluorimager 595 (Molecular Dynamics, Sunnyvale, Calif.). (See FIG. 5).

Other Reference Publication (11):
Rehman, F.N., et al., "Immobilization of acrylamide-modified oligonucleotides by co-polymerization", Nucleic Acids Research, 27(2):649-655 (1999).

CLAIMS:

16. The method of claim 5, wherein the denaturant used for denaturing the double-stranded amplification molecules is denatured using at least one of the following denaturants selected from the group consisting of: high temperature, high pH, organic solvent, chaotropic agent and combinations thereof.

17. The method of claim 5, wherein the material composition of the solid support is selected from the group consisting of: plastic, glass, silica, nylon, metal, metal alloys, polyacrylamide, polyacrylates, crosslinked-dextran and combinations thereof.